

01 NOV 1992

AD-A259 521



MENTATION PAGE

Form Approved
OMB No. 0704-0188

2

ADDRESS TO ORDER: Your order should include the title of the report, the number of copies, and the name of the person to whom the report should be sent. Send orders to the Superintendent of Documents, Government Printing Office, Washington, DC 20540. For information concerning the availability of this report, contact the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

REPORT DATE
10/28/92

1. REPORT TYPE AND DATES COVERED
Annual Technical 9/91-10/92

4. TITLE AND SUBTITLE

Measuring Variation in Ecosystem Sensitivity to Stress

5. FUNDING NUMBERS

AFOSR-91-0379

2312-AF

61102F

6. AUTHOR(S)

John Cairns, Jr., Paul V. McCormick, Eric P. Smith

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University Center for Environmental & Hazardous Materials
Studies
Virginia Tech
Blacksburg, VA 24061-0415

8. PERFORMING ORGANIZATION
REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Air Force Office of Scientific Research/NL
Bolling AFB
Washington, D.C. 20332-6448
Dr. Kozumbo

AFOSR TR- 2 2003

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release;
distribution unlimited.

DTIC
ELECTE
JAN 27 1993
S C D

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The objectives of this study were to develop a method for measuring biological responses to stress in situ and to use this method to evaluate the extent to which stream ecosystems vary in their sensitivity to anthropogenic stress. Chemical-diffusing substrates were constructed from tissue culture flasks and ceramic tiles, which provided a surface that was suitable for the growth of stream microorganisms and porous to most chemicals. Static laboratory tests showed that many common classes of chemicals diffused through these substrates in a highly predictable manner. A laboratory stream study provided further evidence for repeatable diffusion rates and allowed for the standardization of a sampling protocol for characterizing microbial community responses to stress. Two field studies were conducted using the diffusers to evaluate interecosystem variation in stream microbial responses to experimental gradients of sulfuric acid and chlorine. Data analyses from these experiments are ongoing, but indicate that predictable responses to such gradients can be obtained in situ and analyzed using conventional statistical techniques. The protocol developed during the first year of this study has broad applications for both basic and applied research in environmental science.

14. SUBJECT TERMS

15. NUMBER OF PAGES

22

16. PRICE CODE

17. SECURITY CLASSIFICATION
OF REPORT

(u)

18. SECURITY CLASSIFICATION
OF THIS PAGE

(u)

19. SECURITY CLASSIFICATION
OF ABSTRACT

(u)

20. LIMITATION OF ABSTRACT

(u)

Measuring Variation in Ecosystem Sensitivity to Stress

Annual Technical Report

to the

Air Force Office of Scientific Research

Grant AFOSR-91-0379

October 28, 1992

John Cairns, Jr.

DTIC QUALITY INSPECTED 8

Paul V. McCormick

and

Eric P. Smith

University Center for Environmental
and Hazardous Materials Studies

Virginia Polytechnic Institute
and
State University

Blacksburg, Virginia 24061-0415

Accession For	
NTIS	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

03 NOV 1992

93-01468



93

1 26 1990

TABLE OF CONTENTS

Abstract	2
Introduction	3
Objectives	4
Section I: Developing a Test System	4
Methods	4
Chemical Diffuser Design	4
Metabolism Chamber Design	5
Laboratory Characterization of Diffusion Patterns	6
Laboratory Characterization of the Performance of Field Methods	6
Results	9
Laboratory Characterization of Diffusion	9
Laboratory Investigation of the Performance of Field Methods	11
Section II: Evaluating Ecosystem Sensitivity to Stress	13
Methods	13
Site Selection and Experimental Objectives	13
Field Assessment Protocol	14
Preliminary Results	15
Conclusions	17
Literature Cited	18
Figures	20
Appendix A: List of Manuscripts and Presentations	22

ABSTRACT

During the first year of this study, a method for measuring aquatic ecosystem responses to stress *in situ* was refined and subjected to laboratory and field evaluations. Chemical diffusing substrates were constructed using tissue culture flasks and ceramic tiles, which served as substrates for aquatic microbial (bacterial, algal, and protozoan) growth and a porous medium for chemical diffusion. Laboratory diffusion studies were conducted using several chemicals to evaluate the accuracy and precision with which specific chemical dosing regimes could be achieved. An additional experiment was conducted to evaluate microbial community responses to copper using this diffuser system under controlled laboratory conditions in artificial streams. Laboratory studies indicated that predictable rates of delivery could be achieved and that communities also responded in a predictable fashion to dosing. Field studies were conducted to evaluate microbial responses to acid and chlorine stress *in situ* and the degree to which stream ecosystems varied in their sensitivity to these stressors. Analysis of data from these studies is ongoing and will be communicated in future peer-reviewed manuscripts. Initial results indicate that the chemical diffusing system allows for extremely precise characterization of microbial responses to stress in natural ecosystems and that these responses can be evaluated using conventional statistical models.

INTRODUCTION

This report summarizes research conducted during the first year of the grant AFOSR-91-0379 entitled "Measuring Variation in Ecosystem Sensitivity to Stress." The purpose of this research was to evaluate the extent to which natural ecosystems vary in their response to common chemical stressors. Ambient water quality criteria largely adhere to the principle that aquatic ecosystems exhibit similar sensitivities to anthropogenic stress. While variation in specific chemical factors (e.g., hardness) are considered in deriving regulatory criteria for specific ecosystems, there has been no rigorous evaluation of the hypothesis that certain ecosystems are generally more sensitive to anthropogenic insults than others. This study attempted to test this hypothesis for flowing-water ecosystems, which are the primary receiving systems for society's waste (Cairns and Dickson 1977).

The microbial community was used in this study as an indicator of aquatic ecosystem sensitivity to chemical stress. This community comprises the bulk of the biomass in all aquatic ecosystems and is responsible for much of the energy flow and nutrient spiralling within aquatic systems, thus making it a good predictor of changes in ecosystem operation in response to stress. These communities were developed *in situ* on introduced substrates that diffused specific chemicals at measurable rates, thus allowing for changes in microbial community structure and function to be quantified as a function of chemical dose. This approach to evaluating ecosystem response to stress represents a major advancement over laboratory testing procedures since

responses can be quantified under ambient environmental conditions. Furthermore, the chemical-diffusing substrates allowed for dose response relationships to be determined in natural ecosystems without releasing substantial quantities of toxic materials into the ecosystem, thus avoiding unwanted environmental impacts.

OBJECTIVES

The objectives of our three year proposal were to:

- 1) standardize a method for quantifying microbial community responses to stress *in situ*;
- 2) examine variation in sensitivity to common chemical stressors among reference, or minimally-impacted, ecosystems;
- 3) examine variation in sensitivity to a novel chemical stress among ecosystems subjected to different degrees of ambient stress.

The following report details the methods and findings of the first year of the study. The first section details laboratory studies designed to standardize the test system. A second section reports progress on field experiments conducted to measure variations in biological responses to stress among reference ecosystems.

SECTION I: DEVELOPING A TEST SYSTEM

Methods

Chemical Diffuser Design. Chemical-diffusing substrates were constructed by sealing unglazed, 4-inch square, low fire

(cone 05, 1062°C) terra-cotta clay tiles over square holes cut in polystyrene tissue culture flasks using silicone sealant (Figure 1). The substrates were inverted and allowed to rest on clean glass plates covered with a film of commercial, liquid, dishwashing detergent while the silicone sealant cured. The resulting, flat rim allowed metabolism chambers (see below) to be securely sealed over the chemical-diffusing substrates, enclosing a volume of water for the measurement of periphyton community function.

Metabolism Chamber Design. Light and dark chambers were necessary for the characterization of the respiration rate and primary productivity of periphyton communities growing on the chemical-diffusing substrates. Sixteen, identical acrylic chambers, each with a total volume of 560 mL, were constructed (Figure 2). Silicone gaskets were attached to the chambers' flat bases to allow them to be sealed onto the chemical diffusers using rubber bands. Seven of these chambers were covered in black electrical tape, which effectively absorbed incident light and allowed community oxygen consumption to be measured in the absence of photosynthesis. The tubing, pumps, and syringes of the dark chambers were not altered; instead, they were covered with a reflective tarp during dark incubations in situ. The remaining chambers served as light chambers for the measurement of net primary production.

Miniature, submersible pumps (Edmund Scientific) maintained flow across the periphyton mats during light and dark incubations. A plastic syringe, placed in-line with the pump, allowed aliquots of water to be removed during light and dark

incubations without introducing air into the chamber (Figure 2). Air remaining in the system after sealing it to the substrates was removed by opening the screw cap on top of the chamber and depressing the water-filled syringe. A flow interrupter in the syringe insured proper mixing in this portion of the chamber.

A power supply capable of simultaneously running fourteen, miniature, submersible pumps was constructed (Figure 3). Direct current from a rechargeable Dura Power II marine battery (Delco Remy), capable of supplying 85 amperes for 80 minutes, had to be modulated with a square wave generator and a variable transformer to yield a 16 volt, alternating current to insulated pairs of alligator clamps, wired in parallel. The whole circuit was housed in a weather-proofed wooden box.

Laboratory Characterization of Diffusion Patterns. The diffusion patterns of several toxicants were characterized in the laboratory in glass, static diffusion tanks (Figure 4). Chemical diffusers were filled with known concentrations of aqueous toxicant, capped, rinsed, and placed into the glass tanks containing either 1 L (sulfuric acid diffusion experiment) or 2 L (all other diffusion experiments) of distilled, deionized water. Diffusion of toxicant in the static diffusion tanks was periodically monitored by initially renewing the overlying water and then removing aliquots of water at intervals over a 24 hour period for analysis of toxicant concentration. The diffusion patterns of sulfuric acid, zinc sulfate, sodium hypochlorite, and the aquatic herbicide diquat were analyzed using this protocol.

Laboratory Characterization of the Performance of Field Methods. Existing, flow-through, artificial streams at the

Ecosystem Simulation Laboratory on the Virginia Tech campus were slightly modified to allow the performance of the chemical-diffusing substrates and recirculating chambers to be tested (Fig. 5). Periphyton covered rocks taken from the New River, Virginia, were placed in the headbox and served as the source of colonizing organisms for nine artificial streams. Carbon dechlorinated, municipal water, which is derived from the New River, entered the headbox, flowed around the periphyton covered rocks, and flowed into each artificial stream, along with any immigrating organisms, at a rate of 1 L per minute. Current was maintained in the streams with rotating paddle wheels, and water exited the streams through standpipes, which served to establish a 58 L volume of water in each stream.

Six chemical-diffusing substrates, filled with filtered (Nucleopore 0.2 μ m filtration cartridge), dechlorinated tap water, were secured to the bottom of each stream using velcro and periphyton growth was allowed to proceed for 29 days. At the end of this colonization period, one randomly selected substrate was taken out of each stream, and the periphyton was scraped off the clay surface of the substrate and homogenized in a known volume of water. Subsamples were removed from the homogenate for structural analysis (e.g., ash-free dry weight, chlorophyll a, and diatom species richness) using standard methods (APHA et al. 1989).

On day 30, four randomly selected streams were assigned to four different levels of copper (II), which were maintained by dripping known solutions of cupric chloride into the streams at known rates; these were referred to as streams dosed through the

water column. Substrates from four streams, randomly selected from the remaining five streams, were filled with different concentrations of cupric chloride and referred to as streams dosed using the chemical diffusers. No copper was delivered to the remaining control stream. Water in all flasks in the streams dosed through the water column and the control stream was replaced with filtered stream water to control for sloughing of the periphyton mat, which might have occurred when the substrates were filled with toxicant solution and returned to the streams. Qualitatively, however, there was no observed loss of biological material from the chemical diffusers during these manipulations.

After a one week exposure period, one randomly selected substrate was removed from each stream for analysis of structural parameters and three randomly selected substrates were manipulated in situ for characterization of community ammonia uptake rate, net oxygen production, and oxygen consumption in the dark.

Ammonia uptake was assessed by incubating periphyton communities in a 500 ug/L solution of ammonium (made with ammonium chloride and stream water) for 35 minutes. This concentration of ammonium was chosen to be below toxic levels but high enough to be readily detectable, using the phenate method (APHA, 1989), at the beginning and end of the incubation period. The national acute criterion for unionized ammonia is 142.9 ug/L at the temperature and pH (7.35 and 18°C, respectively) of the artificial stream system, and only one percent of a 500 ug/L solution of ammonium is in the more toxic, unionized form under these conditions (USEPA, 1985).

Community net primary productivity was measured as oxygen production in communities incubated for two hours under ambient conditions of temperature (18°C) and incident light ($40 \text{ mE/m}^2/\text{sec}$). Stream water, through which nitrogen gas had been bubbled in order to reduce the dissolved oxygen concentration, was used to fill the recirculating chambers. Changes in oxygen content were calculated by measuring dissolved oxygen in the chambers at the beginning and end of the incubation periods using the azide modification method (APHA, 1989). The respiration rate of communities exposed to different levels of copper through the water column or via chemical diffusers was similarly measured as oxygen consumption by communities incubated in dark chambers for 90 minutes.

Results

Laboratory Characterization of Diffusion. Chemical diffusers containing solutions of sulfuric acid demonstrated an initial lag phase, characterized by a relatively low rate of diffusion. This was followed by a sustained phase of increased diffusion, measured as a decrease in water column pH (Figure 6). During this experiment, distilled water was used in the static diffusion tank for the first five days. However, tap water was used on the sixth and all subsequent sampling days in order to stabilize the pH readings. This resulted in the shift in initial pH (at hour 0) seen in Figure 6. The apparent leaching of a basic component from the clay tiles of the controls (i.e., substrates filled with distilled, deionized water) resulted in an increase in water column pH even though the clay substrates were

soaked in distilled water for two days prior to this experiment.

The diffusion of zinc into the water column in the static diffusion tanks was also sustained at a given level for eleven days, following an initial phase of greater diffusion (Figure 7). The results of both the diffusion experiment using sulfuric acid and the experiment using zinc sulfate demonstrate that there is a high degree of replicability in diffusion rates between chemical-diffusing substrates. In other words, the physical and chemical make-up of the tiles appears to be fairly consistent, based on these results.

Sodium hypochlorite (commercial bleach) also diffused across the clay tiles at an elevated rate on the first day (Figure 8). Subsequently, the diffusion of hypochlorite occurred at a lower level for thirteen days. There appeared to be a slightly greater degree of variability in hypochlorite diffusion, relative to the diffusion of zinc. However, chlorine is known to be volatile, and hypohalite chemistry is very complex (Greenwood and Earnshaw, 1986). Thus, this diffusion experiment will be repeated under modified experimental conditions (e.g., with reduced head space and lids for the static diffusion tanks).

The diffusion of aqueous diquat dibromide, an organic, aquatic herbicide, was also assayed. However, diquat did not diffuse across the chemical-diffusing substrates at any of the concentrations used.

The results of the laboratory diffusion experiments conducted thus far indicate that the toxicants proposed for use in field manipulations (e.g., sulfuric acid and chlorine) can be delivered for a sustained period of time with a high degree of

replicability.

Laboratory Characterization of the Performance of Field Methods. Water column concentrations of copper during the one week exposure period in the artificial streams dosed through the water column and the artificial streams dosed using the chemical diffusers are shown in Figures 9 and 10, respectively. The initial spikes in copper concentration in the four streams dosed through the chemical-diffusing substrates resulted from unpreventable overflow of the copper solutions onto the tissue culture flasks. After the diffusers were filled with the appropriate solutions of copper the polystyrene portions of the constructed substrates were rinsed with distilled water before returning them to the artificial streams. This certainly reduced the initial increase in water column copper in these artificial streams but did not totally eliminate it. Without exception, cupric chloride was prevented from overflowing onto the established periphyton communities by slightly tipping the substrates while being filled with toxicant solution.

Figure 11 illustrates a highly significant linear relationship between bioconcentration of copper in the periphyton mat and average water column copper concentration ($p < 0.0001$; $r^2 = 0.998$). In the range of cupric chloride solutions used, bioconcentration of copper was also a linear function of the concentration of copper inside the chemical diffuser ($p = 0.0008$ and $r^2 = 0.984$ for the linear model) as shown in Figure 12. Thus, copper (II) can be delivered in a very predictable manner to periphyton communities using chemical-diffusing substrates.

Reductions in water column ammonia in recirculating chambers

sealed over periphyton communities were very slight (data not shown). However, no reduction in water column ammonia occurred at the highest levels of copper bioconcentration, and the greatest level of ammonia uptake occurred in the control stream. Thus, copper appears to have impaired this functional parameter. Ammonia uptake holds promise as a functional endpoint, which is measurable using the recirculating chambers; however, a longer incubation period (e.g., one hour) should be employed in future ammonia uptake experiments.

Measurable oxygen production occurred during productivity incubations at the low incident light intensities received by the laboratory streams (i.e., $40 \mu\text{E}/\text{m}^2/\text{sec.}$). This level is comparable to the midday, summer, ambient light intensity of a heavily shaded stream. The observed reduction in chlorophyll-specific net primary productivity with increasing copper bioconcentration (Figure 13) is consistent with the findings by Stauber and Florence (1987) that the copper (II) ion inhibits photosynthesis in the marine diatom Nitzschia closterium and the freshwater green alga Chlorella pyrenoidosa. In addition, Figure 14 demonstrates a reduction in the productivity to biomass ratio, indicating a shift to a more heterotrophic community following copper stress. A similar shift was found to occur in complex, lentic microcosms in response to copper (II) (Hedtke, 1984).

Reductions in oxygen concentration in dark chambers during the 90 minute incubations (Figure 15) indicate that the recirculating chambers are sealing well to the substrates and that the submersible pumps are not significantly exchanging chamber water with stream water or otherwise aerating the

enclosed water. As with net primary productivity, the respiration of the periphyton communities growing on the chemical-diffusing substrates was impaired by copper.

The results of these functional assays provide graphical evidence that, under the conditions of this laboratory experiment (i.e., low light levels and incubations lasting no longer than two hours) changes community net primary productivity and respiration can be assessed by determining oxygen changes in the constructed chambers.

SECTION II: EVALUATING ECOSYSTEM SENSITIVITY TO STRESS

Methods

Site selection and experimental objectives. Five minimally-impacted streams in the southwestern Virginia area were identified for experimentation during fall 1991. Selection criteria included: 1) a lack of point source discharges; 2) minimal nonpoint pollution impacts, as evidenced by the presence of an intact riparian zone and surrounding land use patterns; 3) macroinvertebrate community composition based on EPA-approved sampling protocols (Plafkin et al., 1989). Streams were selected from watersheds draining both the Blue Ridge and Appalachian mountain ranges and, thus, exhibited differences in certain chemical characteristics (e.g., pH, alkalinity, etc.) resulting from geological differences in the underlying substrata. Three tributaries draining the Roanoke River (Blue Ridge) were selected for study: 1) Goose Creek; 2) Rock Creek; 3) Bradshaw Creek. Two tributaries draining the New River were also selected: 1) Kimberling Creek; 2) John's Creek.

Separate experiments were conducted to compare the response of the microbial community in the five streams to two common chemical inputs: 1) sulfuric acid; 2) chlorine. The general methodology is described below followed by the preliminary results of each trial. At the time this report was prepared, data analyses were still in progress. Initial data are presented in order to verify that discernible trends in microbial community response to stress could be elucidated using our experimental protocol. Peer-review manuscripts are being prepared concurrent with the analysis of field data from year 1. Once completed, copies of these manuscripts will be sent to the Air Force Office of Scientific Research. A list of manuscripts in preparation and oral presentations that have resulted from the first year of the study are given in Appendix A.

Field Assessment Protocol. Chemical diffusing substrates were placed out in the five streams on wooden pallets. Substrates were attached to the pallets using velcro strips. Pallets were securely weighted to the stream bottom using large rocks.

Initially, all substrates were filled with filter-sterilized stream water. Substrates were incubated in the stream for 21 days, by which time noticeable microbial biomass had accumulated on the clay surfaces. Substrates were carefully removed from the streams at this time so that the filtered stream water could be replaced by a toxicant solution. The range of exposure was identical for the acidification (sulfuric acid) experiment and the chlorination experiment. Multiple substrates were filled with one of the following molar concentrations of toxicant

solution: 0, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0.

The attached microbial community was exposed to the toxicant solution for 7 days before sampling to determine the extent of response to the stressor. Two substrates of each concentration were used to determine microbial community response to the toxicant. Primary productivity and respiration measurements were conducted *in situ* using the chambers described in an earlier section of this report. Following completion of these incubations, the community was scraped from the clay surface and preserved in 3% Bouin's fluid. Preserved material was used to prepare quantitative mounts of algal and protozoan cells for analysis of microbial biomass (ash-free dry mass), taxonomic composition (algal) and functional group composition (protozoan).

Toxicant solutions in remaining substrates were replaced with filter-sterilized stream water after 7 days of exposure and incubated in the streams for seven additional days to allow for the microbial community to begin to recover from the stress. Data were collected from these substrates in a manner identical to that used for substrates described above.

Preliminary Results

Data analysis from the chlorine experiment has proceeded smoothly and repeatable, statistically-significant responses have been detected for several biological parameters. The repeatability of community responses to stress is illustrated in Figure 16, which shows the response of community biomass to increasing concentrations of chlorine. Most functional and structural responses to stress exhibited repeatable patterns of response across ecosystems as well, thus allowing for identical

statistical models to be used to characterize community responses in different ecosystems. Thus, while statistical analyses have yet to be completed for field experiments, we are optimistic that valid response comparisons can be made using the *in situ*, experimental technique developed in this study.

CONCLUSIONS

During the first year of this project, we have refined an *in situ* technique for experimentally determining the sensitivity of stream microbial communities to chemical stress. This design includes a standardized protocol for on-site characterization of functional responses (productivity, respiration). This design has been subjected to rigorous standardization in the laboratory and the field. Specific achievements include:

- 1) verifying predictable diffusion rates for a variety of common chemicals;
- 2) verifying a predictable correspondence between rates of chemical diffusion and rates of bioaccumulation in the attached microbial community;
- 3) refining a method for obtaining sensitive measures of community metabolism *in situ*.

Additional field studies have been completed to evaluate community responses to common chemical stressors and determine the degree of variation in sensitivity to anthropogenic stress among stream ecosystems. Preliminary analyses from these experiments indicate that predictable and replicable responses to stress can be obtained *in situ* using the diffusing substrates.

We foresee several applications for this method in environmental research. First, experimental results indicate that this method provides a suitable means for evaluating fundamental hypotheses in environmental biology, including the degree to which ecosystems differ in their sensitivity to different forms of anthropogenic stress. Other applications include the validation of laboratory predictions of chemical

hazard and the verification of longitudinal impacts resulting from single point-source discharges as well as cumulative impacts.

LITERATURE CITED

- American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, Washington, DC.
- Cairns, J., Jr., and K. L. Dickson. 1977. Recovery of streams from spills of hazardous material. In: Recovery and Restoration of Damaged Ecosystems (J. Cairns, Jr., K. L. Dickson, and E. E. Herricks, eds.). University Press of Virginia, Charlottesville, VA. pp. 24-42.
- Greenwood, N. N. and A. Earnshaw. 1990. Chemistry of the Elements. Pergamon Press, New York.
- Hedtke S. F. 1984. Structure and function of copper-stressed aquatic microcosms. Aquatic Toxicology. 5:227-244.
- Plafkin, J. L., M. T. Barbour, K. D. Porter, S. K. Gross, and R. M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish. EPA 444/4-89/001. U.S. Environmental Protection Agency, Washington, D.C.
- Stauber, J. L. and T. M. Florence. 1987. Mechanism of toxicity of ionic copper and copper complexes to algae. Marine Biology. 94:511-519.

United States Environmental Protection Agency. 1985. Ambient
water quality criteria for ammonia. EPA 440/5-85-001.
National Technical Information Service, Springfield, VA.

FIGURES

Figures 1-4: Fig. 1) Chemical-diffusing substrate; Fig. 2) Recirculating chamber for the measurement of community O_2 consumption and production; Fig. 3) Power supply circuit for the miniature, submersible pumps (shown as circles); Fig. 4) static diffusion tank for the laboratory characterization of diffusion patterns.

Figure 5: Artificial stream system used for testing the efficacy of the recirculating chambers in allowing detection of changes in dissolved oxygen due to periphyton community metabolism.

Figure 6: Diffusion of sulfuric acid on days 2 (open circles), 4 (open triangles pointing down), 6 (open squares), 8 (open triangles pointing up), and 12 (open diamonds) at the following concentrations: 0 M (Fig. 3.1), 0.05 M (fig. 3.2), and 0.1 M (Fig. 3.3). Means ($n=7$) \pm one standard error are shown.

Figure 7: Diffusion of zinc sulfate on days 1 (open circles), 2 (open triangles pointing down), 4 (open squares), and 11 (open triangles pointing up). The figure shows the diffusion of $0.5 \text{ M ZnSO}_4 \cdot 7\text{H}_2\text{O}$ along with a distilled, deionized water control (data points along the x axis). Means ($n=4$) \pm one standard error are shown.

Figure 8: Diffusion of sodium hypochlorite on days 1 (open circles), 2 (open triangles pointing down), 4 (open squares), 6 (filled circles), 8 (filled triangles pointing down), and 13 (filled squares) at the following concentrations: 0 M (fig 5.1), 0.01 M (fig. 5.2), 0.05 M (fig. 5.3), 0.1 M (fig. 5.4), and 0.5 M (fig. 5.5). Means ($n=2$) \pm one standard error are shown.

Figure 9: Water column copper concentrations maintained for one week in the artificial streams dosed through the water column (streams 5, 7, 2, and 9) and the control (stream 3).

Figure 10: Water column copper concentrations resulting from use of the chemical-diffusing substrates in artificial streams. Concentrations listed in the legend are the nominal concentrations of copper (II) used in the chemical diffusers.

Figure 11: Relationship between periphyton copper bioconcentration and water column copper concentration. The parameter estimates, r^2 , and the significance level for the linear model are shown in the upper left hand corner of the figure.

Figure 12: Relationship between periphyton copper bioconcentration and the concentration of copper (II) used in the chemical-diffusing substrate. The parameter estimates, r^2 , and the significance level for the linear model are shown in the upper left hand corner of the figure.

Figure 13: Net primary productivity, measured as community oxygen production per unit chlorophyll per milli-Einstein of photons, versus copper bioconcentration.

Figure 14: Net primary productivity, measured as community oxygen production per unit biomass over time, versus copper bioconcentration.

Figure 15: Community respiration, measured oxygen consumption per unit biomass over time, versus copper bioconcentration.

Figure 16: Community biomass versus molar concentration of chlorine as hypochlorite in diffusing substrates after 7 days of exposure in a natural stream. Triangles are the individual data points, the solid line is the predicted response curve ($p=0.0104$, regression F) and dotted lines are the 95% confidence bands for the predicted relationship.

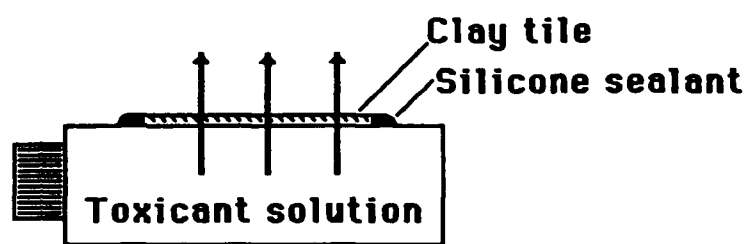


Figure 1 Tissue culture flask

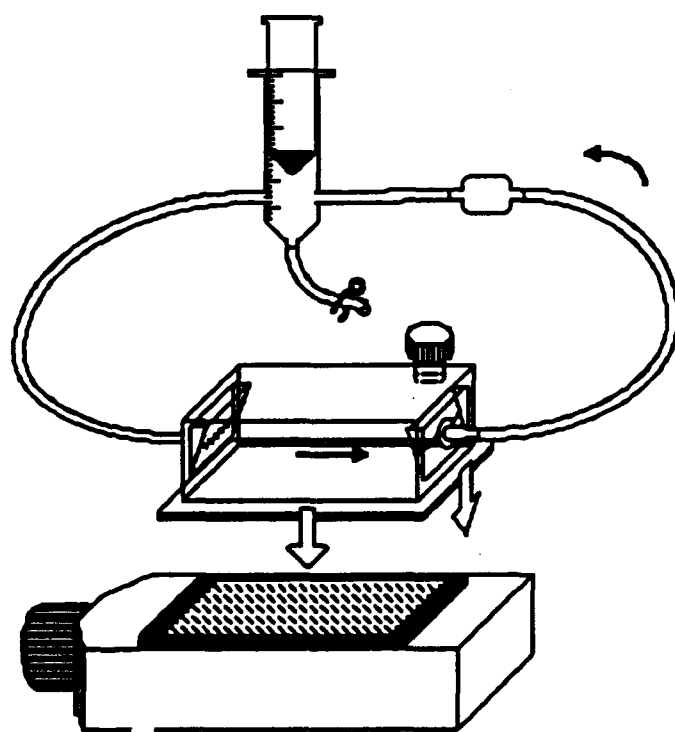


Figure 2

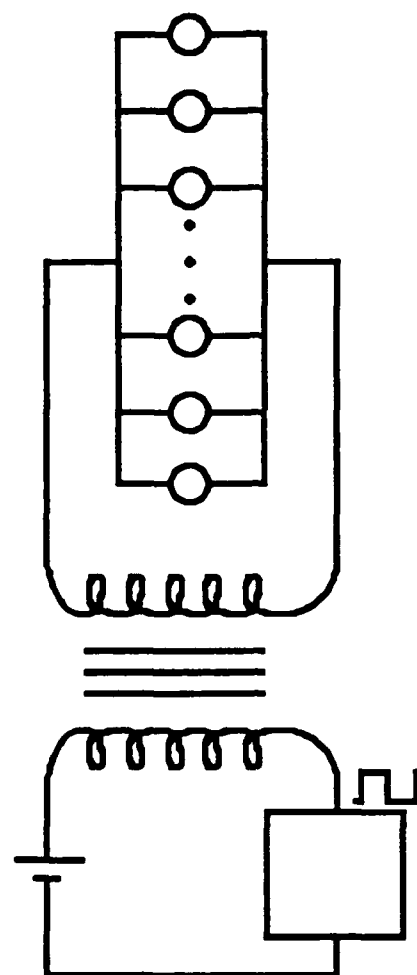


Figure 3

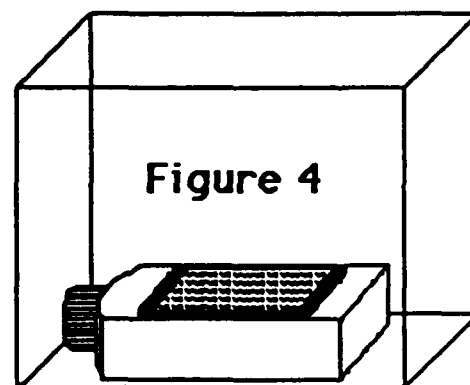
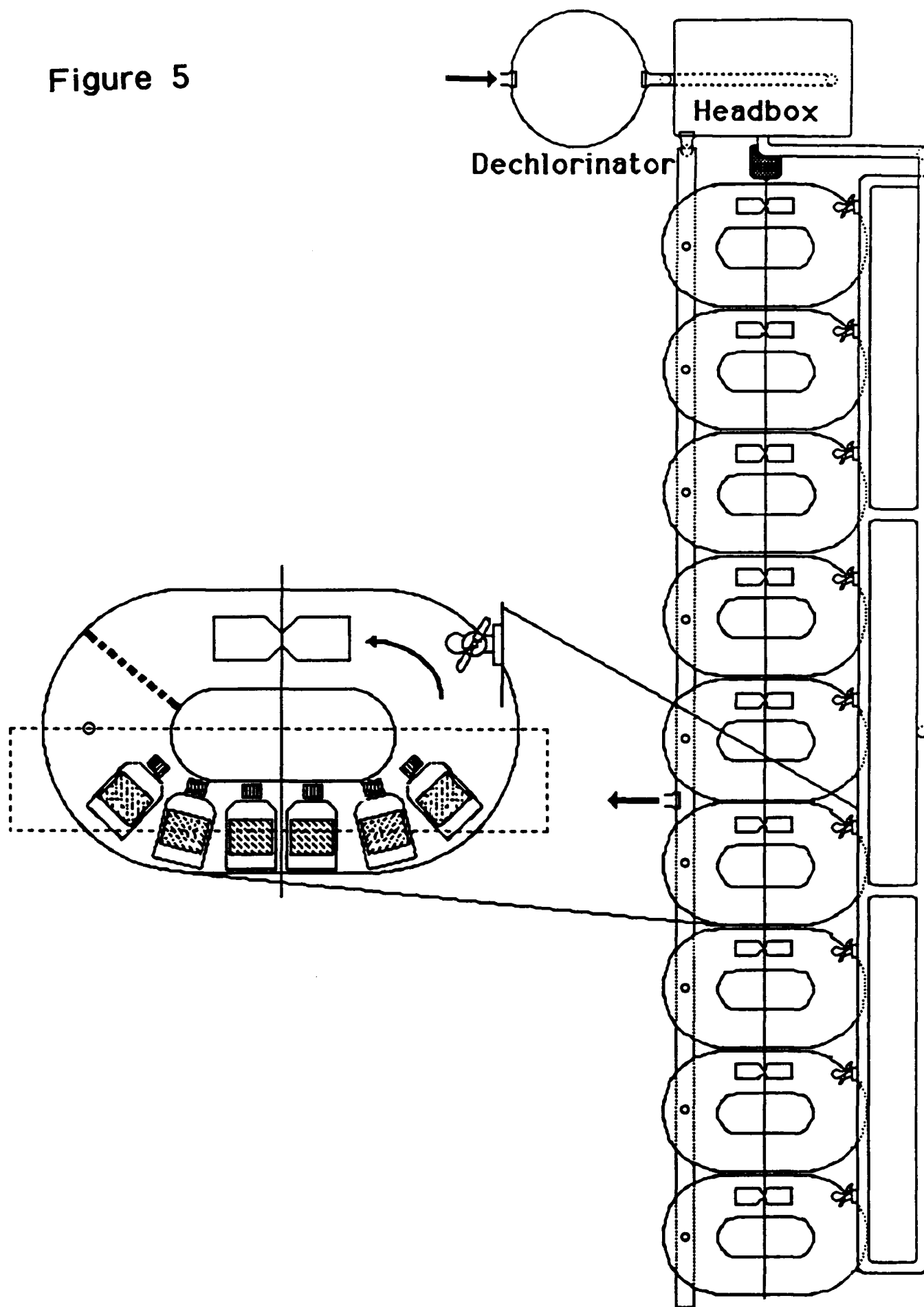


Figure 4

Figure 5



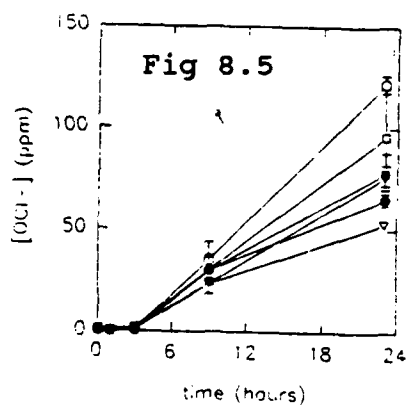
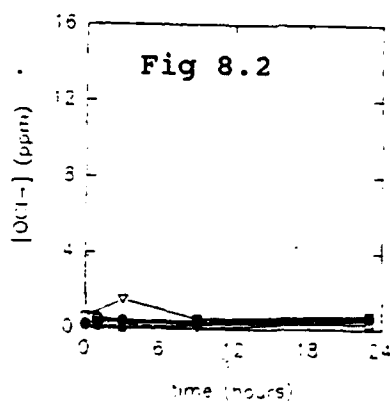
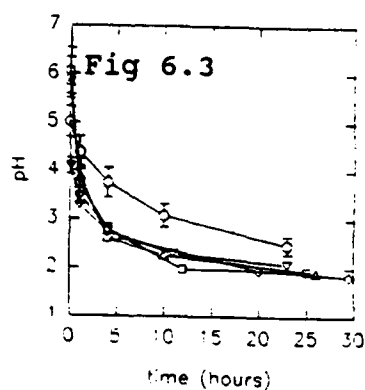
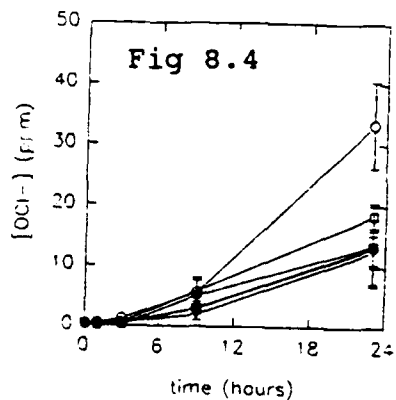
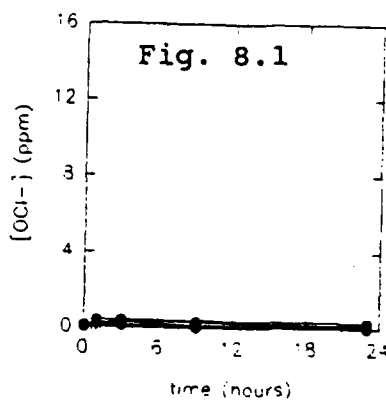
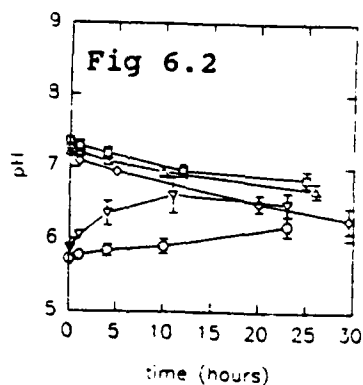
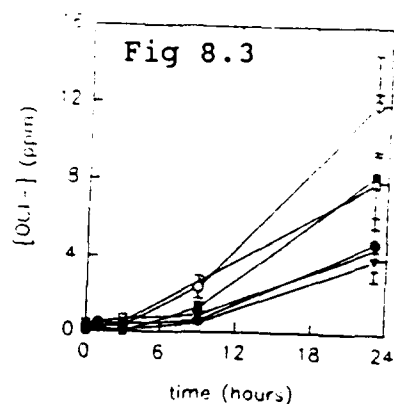
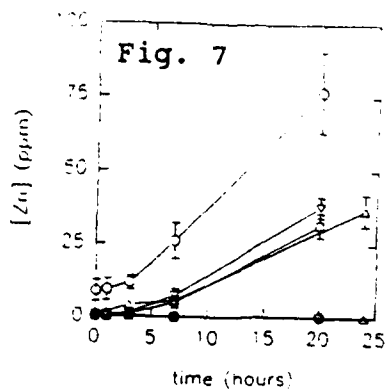
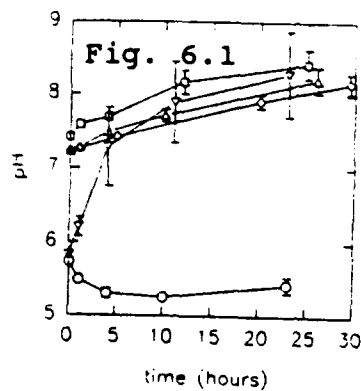


Figure 9

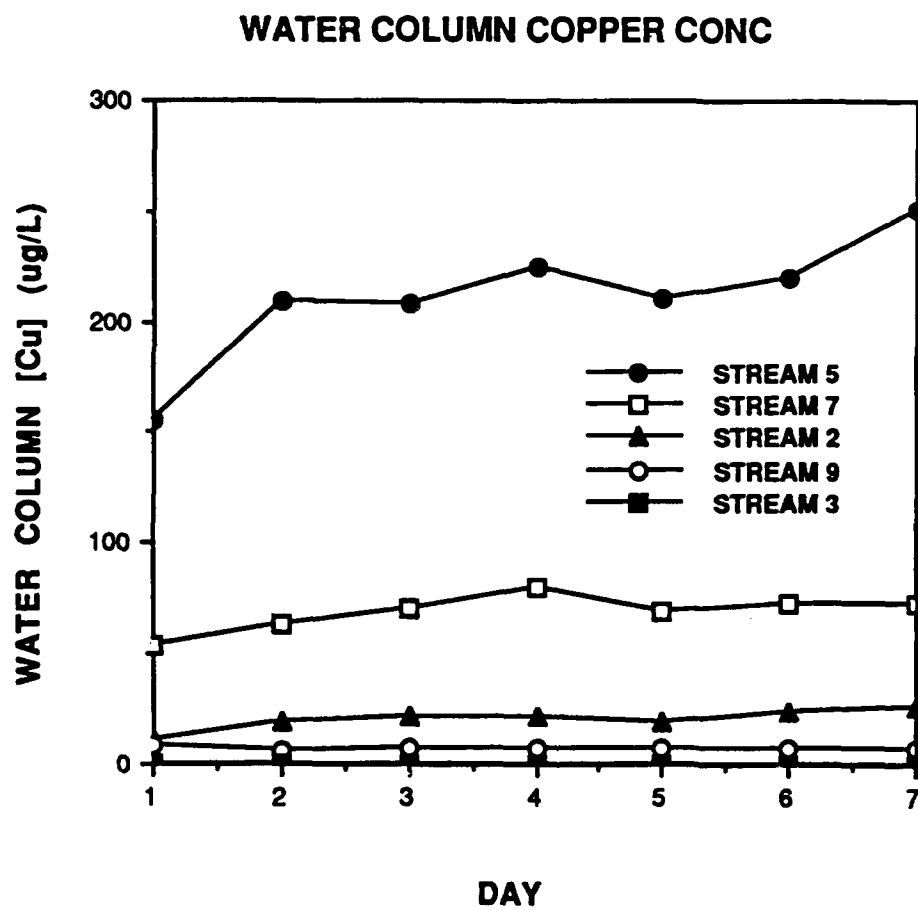


Figure 10

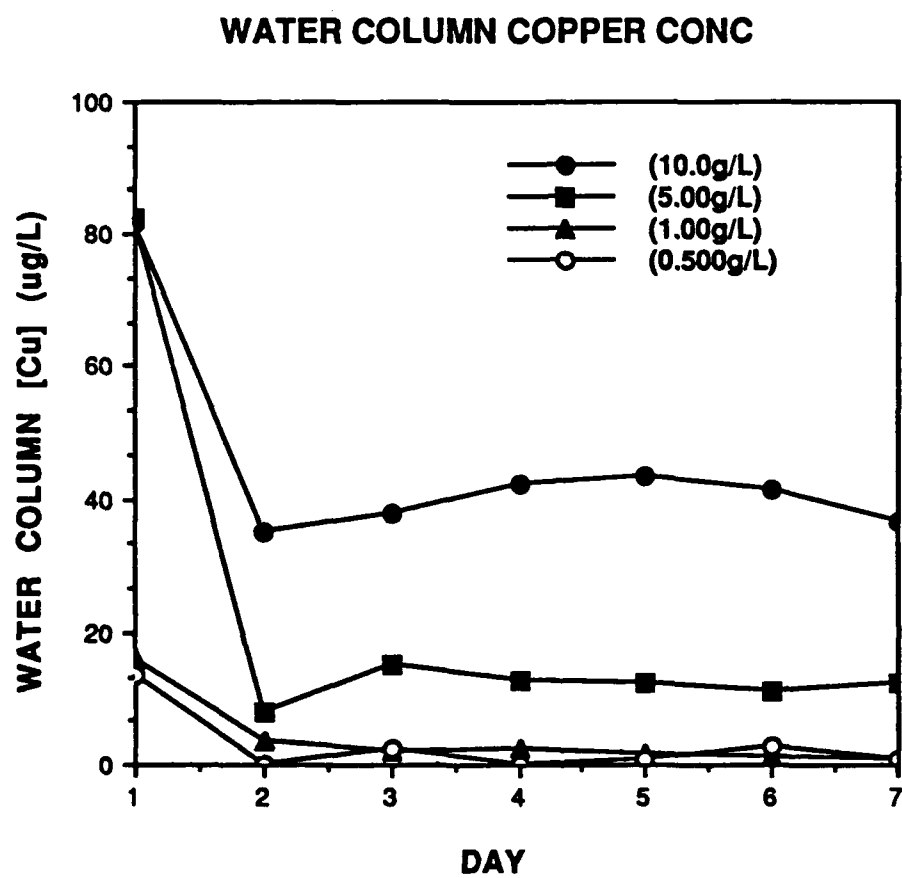


Figure 11

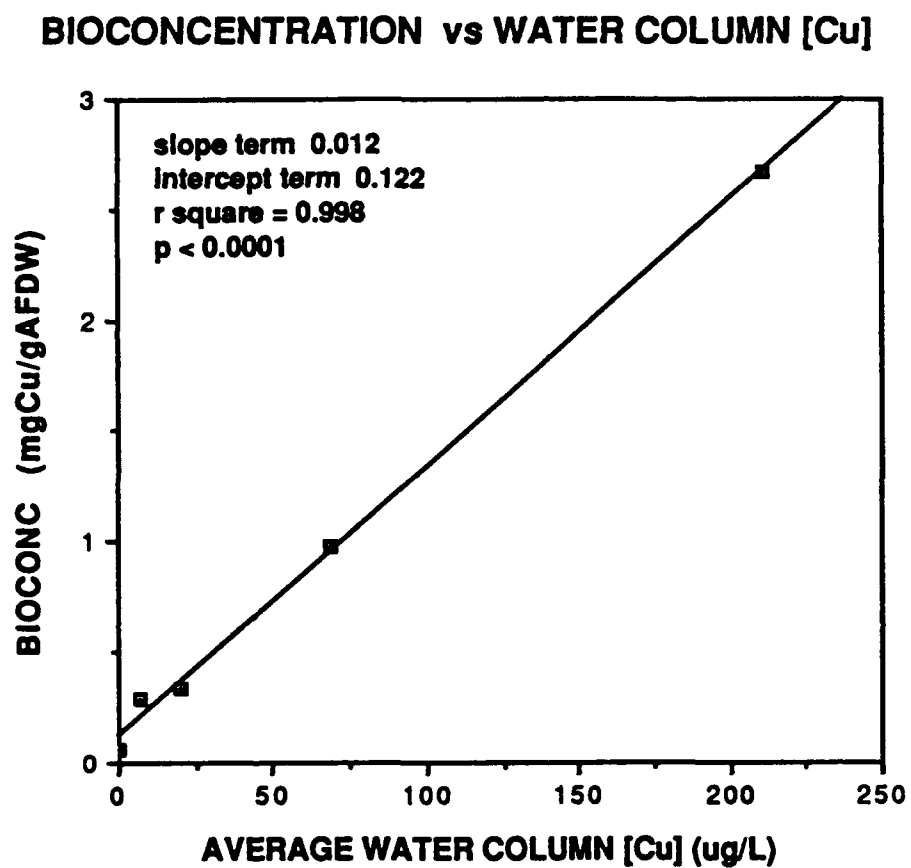


Figure 12

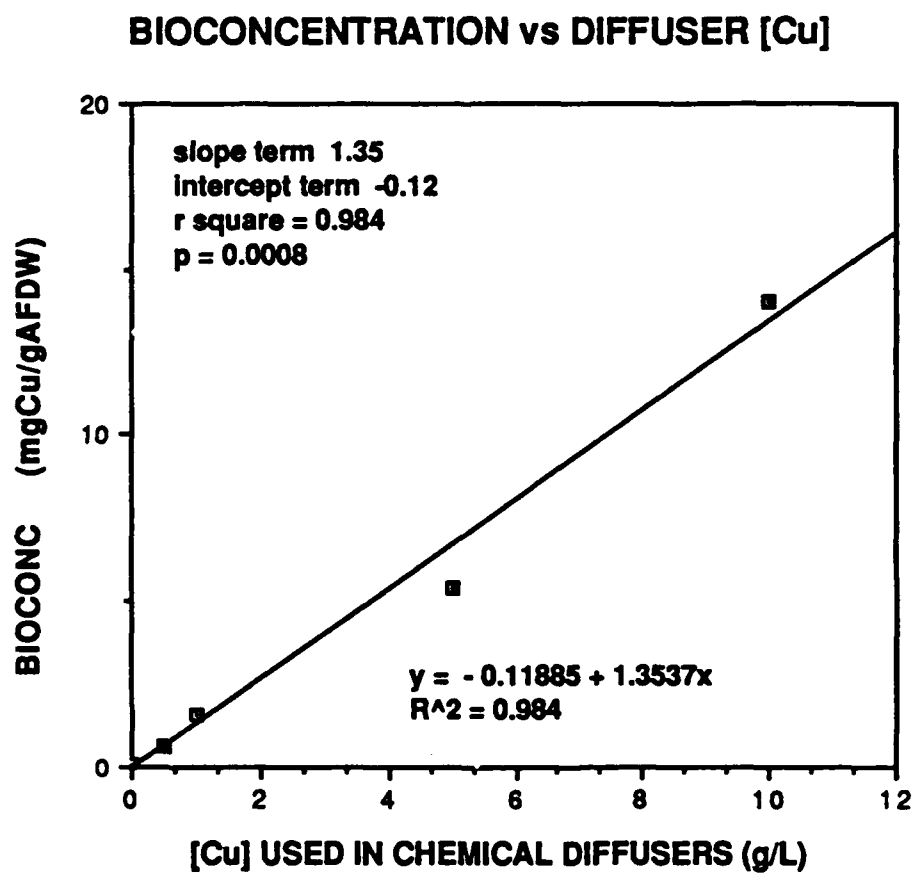


Figure 13

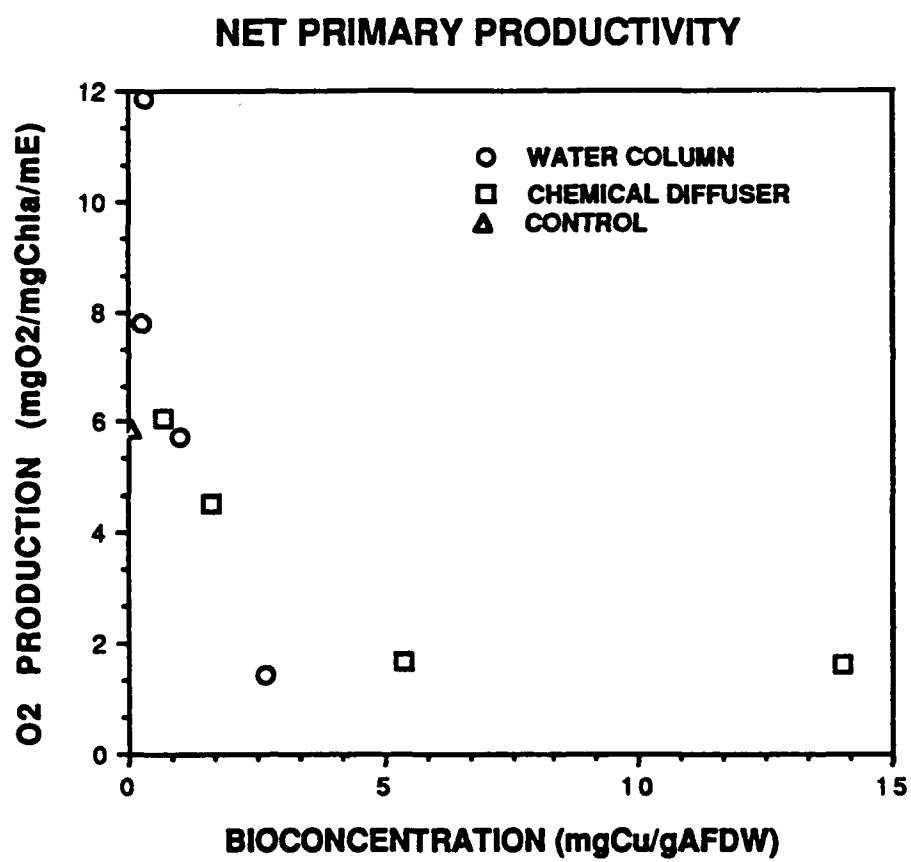


Figure 14

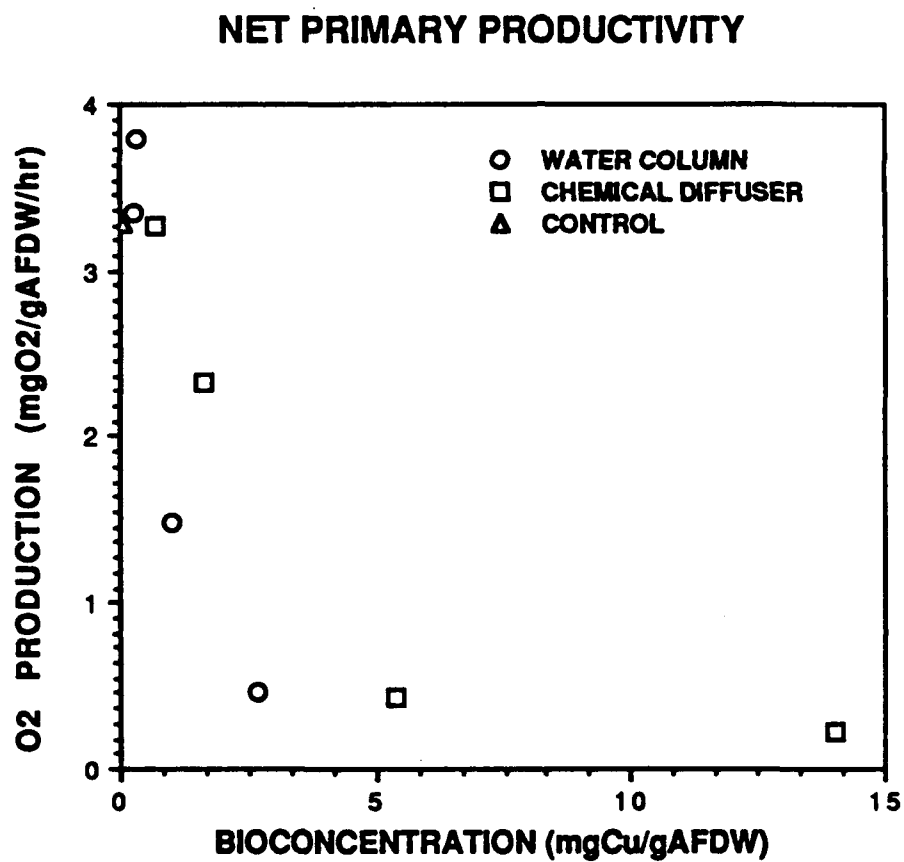


Figure 15

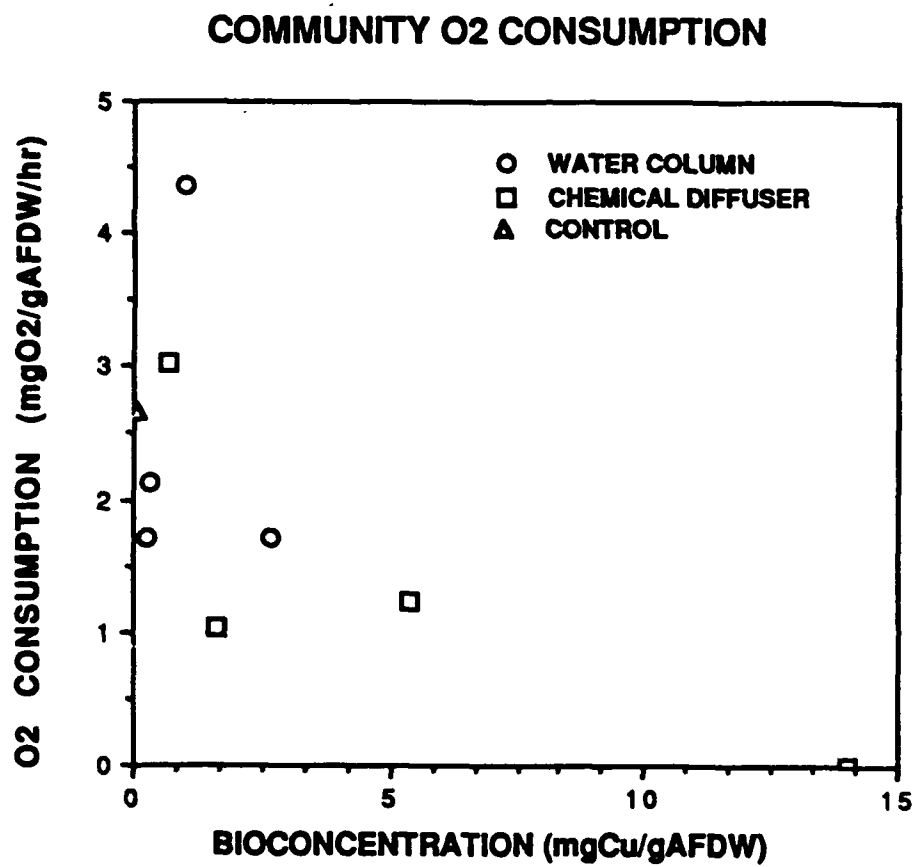
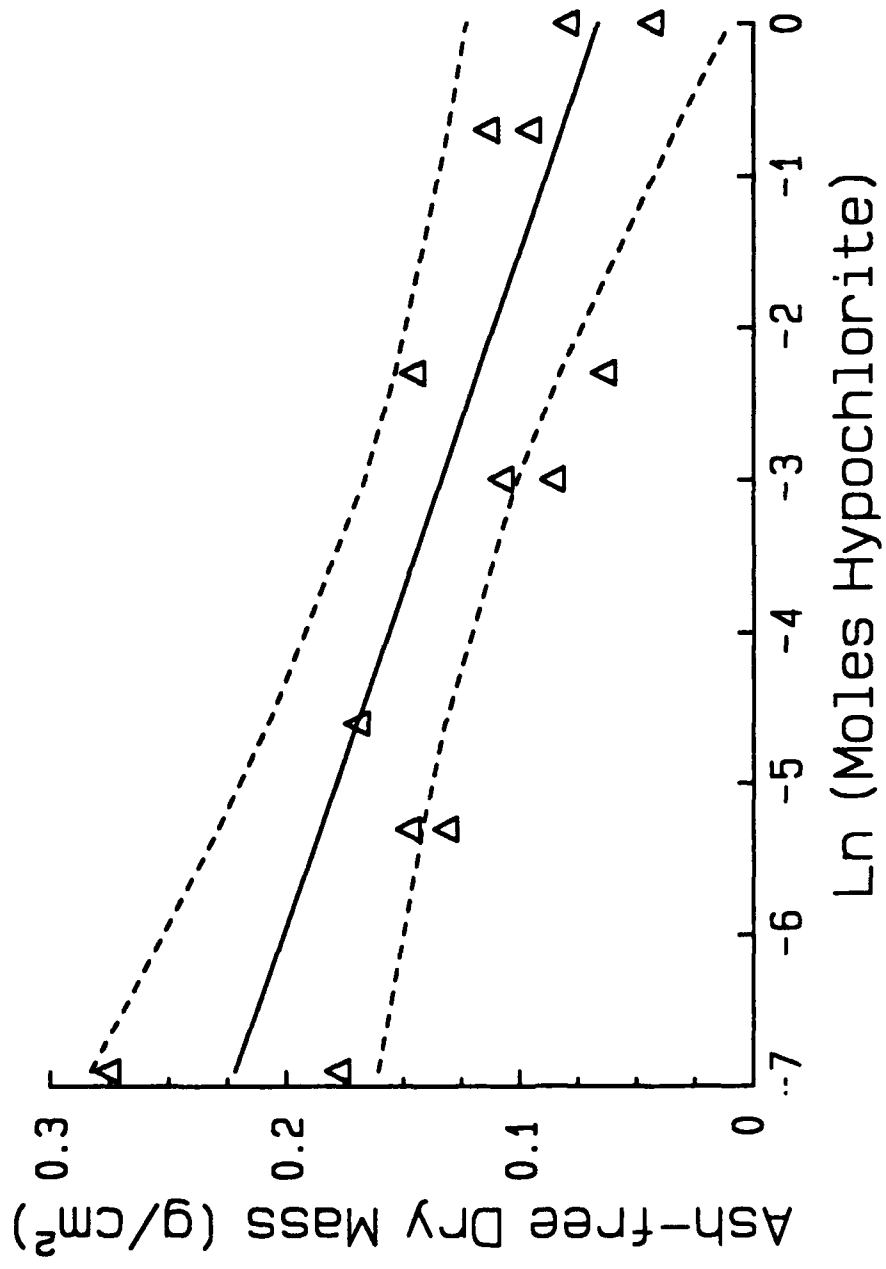


Figure 16



APPENDIX A

I. Manuscripts in preparation

Arnegard, M., P. V. McCormick, and J. Cairns, Jr. In preparation. A field evaluation of the independent and interactive effects of metal and acid contamination from an abandoned mine on stream communities. To Aquatic Toxicology.

Cairns, J., Jr., and P. V. McCormick. In preparation. Developing a field-based capacity for predicting ecosystem sensitivity to stress. To Environmental Professional.

McCormick, P. V., M. Arnegard, and J. Cairns, Jr. In preparation. An *in situ* chemical-diffusing substrate for field validation of laboratory toxicity predictions. To Journal of Freshwater Ecology.

McCormick, P. V., M. Arnegard, and J. Cairns, Jr. In preparation. Measuring variation in stream ecosystem sensitivity to chlorine. To Environmental Toxicology and Chemistry.

II. Oral Presentations

Arnegard, M. E. and J. Cairns, Jr. A new method for assessing the effects of inorganic pollutants on algal communities in streams. Presented at the Third Annual Environment Virginia Symposium, 1992.

Arnegard, M. E., P. V. McCormick, and J. Cairns, Jr. A new method for assessing the effects of toxicants on Aufwuchs communities *in situ* using chemical-diffusing substrates. Presented at the 1992 Annual Meeting of the North American Benthological Society.

Arnegard, M. E., P. V. McCormick, and J. Cairns, Jr. The development of chemical-diffusing substrates for *in situ* periphyton community surveys. Presented at the 1991 Annual Meeting of the Virginia Academy of Science.